A Pilot Investigation of the Relative Toxicity of Indoor and Outdoor Fine Particles: *In Vitro* Effects of Endotoxin and Other Particulate Properties

Christopher M. Long,[1] Helen H. Suh,[2] Lester Kobzik,[2] Paul J. Catalano,[3,4] YaoYu Ning,[2] Petros Koutrakis[2]

[1]Gradient Corporation, Cambridge, Massachusetts, USA; [2]Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA; [3]Department of Biostatistical Science, Dana-Farber Cancer Institute, Boston, Massachusetts, USA; [4]Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts, USA

In this study we assessed the *in vitro* toxicity of 14 paired indoor and outdoor $PM_{2.5}$ samples (particulate matter ≤ 2.5 μm in aerodynamic diameter) collected in 9 Boston-area homes. Samples were collected as part of a large indoor particle characterization study that included the simultaneous measurement of indoor and outdoor $PM_{2.5}$, particle size distributions, and compositional data (e.g., elemental/organic carbon, endotoxin, etc.). Bioassays were conducted using rat alveolar macrophages (AMs), and tumor necrosis factor (TNF) was measured to assess particle-induced proinflammatory responses. Additional experiments were also conducted in which AMs were primed with lipopolysaccharides (LPS) to simulate preexisting pulmonary inflammation such as that which might exist in sick and elderly individuals. Significant TNF production above that of negative controls was observed for AMs exposed to either indoor or outdoor $PM_{2.5}$. TNF releases were further amplified for primed AMs, suggesting that preexisting inflammation can potentially exacerbate the toxicity of not only outdoor $PM_{2.5}$ (as shown by previous studies) but also indoor $PM_{2.5}$. In addition, indoor particle TNF production was found to be significantly higher than outdoor particle TNF production in unprimed AMs, both before and after normalization for endotoxin concentrations. Our results suggest that indoor-generated particles may be more bioactive than ambient particles. Endotoxin was demonstrated to mediate proinflammatory responses for both indoor and outdoor $PM_{2.5}$, but study findings suggest the presence of other proinflammatory components of fine particles, particularly for indoor-generated particles. Given these study findings and the fact that people spend 85–90% of their time indoors, future studies are needed to address the toxicity of indoor particles. *Key words*: alveolar macrophage, cytokines, endotoxin, fine particles, indoor air pollution, $PM_{2.5}$, toxicity, tumor necrosis factor. *Environ Health Perspect* 109:1019–1026 (2001). [Online 26 September 2001]
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Given the fact that people spend 85–90% of their time indoors (*1*), it is widely recognized that a significant portion of total personal exposures to particulate matter (PM) occurs in indoor environments. Indoor particles are composed of both ambient particles, which infiltrate indoors, and nonambient particles, which are generated indoors during the daily activities of home occupants. In a previous paper (*2*), we demonstrated that indoor fine particle concentrations in nine Boston-area study homes were significantly elevated during cooking, cleaning, and other general indoor activities involving combustion (e.g., burning candles) or physical movement (e.g., walking). Indoor source events were typically of short duration, but many were of very high intensity, capable of raising hourly concentrations of $PM_{2.5}$ (particulate matter ≤ 2.5 μm in aerodynamic diameter) by tens to hundreds of micrograms per cubic meter. Furthermore, this and other studies have shown that indoor particle events can substantially modify the size distribution and composition of indoor particles (*2–7*).

Exposures to indoor-generated particles may be highly relevant to public health because of the high frequency of exposure to large short-term events. In fact, concern over the health significance of exposures to peak short-term concentrations has grown due to the findings of several recent studies that short-term ambient PM events are associated with acute health outcomes (*8–13*). Due to differences in size distributions and composition, it is possible that indoor-generated particles may be more or less toxic than ambient particles.

However, given the U.S. Environmental Protection Agency's (U.S. EPA) mandate to regulate ambient air pollution, epidemiologic and toxicologic studies have traditionally addressed only the health impacts of ambient particles. Over 150 epidemiologic studies have reported significant associations between ambient PM levels and excess mortality and morbidity (*14*). Among the adverse health outcomes that have been most strongly linked to ambient PM exposures are cardiopulmonary mortality, symptoms of respiratory and cardiovascular disease, and impaired lung function. Toxicologic studies are ongoing to determine the causal agents and underlying mechanisms for ambient PM health effects (*15,16*).

Due to their low cost and sensitivity, *in vitro* toxicity tests are beginning to be used more widely as exploratory tools in PM toxicologic studies. *In vitro* bioassays have been more extensively used to investigate the toxicologic properties of homogeneous particle mixtures including residual oil fuel ash, urban air particles (UAP), inert titanium dioxide, elemental carbon, and diesel particles (*17–25*). Only very recently have studies reported bioassay data for ambient $PM_{2.5}$ and PM_{10} samples. These studies have demonstrated a variety of biological responses for alveolar macrophages, blood monocytes, and respiratory epithelial cells including cytotoxicity, particle phagocytosis, oxidant production, and production of inflammatory mediators (*21–23,26–28*). Specifically, these studies have provided evidence that particle-bound endotoxin and trace metals contribute to the observed biological activity of ambient PM samples.

Despite the public health implications of indoor particle exposures, only one of these studies reported bioassay findings for indoor particles (*26*). In this study, we used *in vitro* bioassays to investigate the relative toxicity of indoor and outdoor $PM_{2.5}$ that was collected from nine Boston-area homes as part of a large indoor particle characterization study. Similar to previous studies (*23,29*), the bioassays measured the tumor necrosis factor (TNF) released by rat alveolar macrophages (AMs) after exposures to indoor and outdoor particles. We chose TNF as the measurement end point because it is a potent proinflammatory mediator in the lung that has been shown to play a crucial role in the recruitment and activation of numerous inflammatory cells (*30*). To simulate preexisting pulmonary inflammation such as that which

Address correspondence to C.M. Long, Gradient Corporation, 238 Main Street, Cambridge, MA 02142 USA. Telephone: (617) 395-5000. Fax: (617) 395-5001. E-mail: clong@gradientcorp.com

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might exist in sick and elderly individuals, we also conducted bioassays using macrophages that were primed with lipopolysaccharides before the particle exposures.

Materials and Methods

Study design. As described previously, we sampled nine nonsmoking Boston-area homes for 1 or 2 week-long periods during spring–summer and fall–winter 1998 (*2*). All homes were located within 30 miles of downtown Boston in suburban neighborhoods. Study homes were typical of homes in New England, a region in the United States with four distinct seasons including cold winters and warm summers. Windows and doors were predominantly kept closed for the winter months as well as the majority of the spring and fall sampling periods. During the winter months, five of the nine homes were heated with oil, whereas the remaining four had natural gas heating systems. Similarly, five homes had radiant heat and four had forced-air heat. During the summer months, home occupants typically opened windows and doors to promote air circulation. The major exception was Home FOX1, which relied upon a central air-conditioning system during the summer months, including its July sampling event.

Five of the nine study homes were sampled during each of two seasons. All homes were sampled a minimum of 6 consecutive days on each sampling occasion, with most homes sampled for at least 7 days and several for longer periods. Table 1 summarizes the locations, sampling dates, and sampling duration for each study home.

Toxicity sample collection. Harvard Impactors (HI; Air Diagnostics and Engineering, Inc., Harrison, ME) were used to collect indoor and outdoor $PM_{2.5}$ samples for the bioassays. To obtain a sufficient mass of particles, these samplers were operated continuously for the duration of sampling at each home. Hence, one indoor and one outdoor sample were collected during each sampling period for a total of 14 indoor and 14 outdoor samples.

$PM_{2.5}$ HIs were operated at a flow rate of 10 L/min according to previously documented specifications (*31,32*). Flow rates were measured every 12 hr using calibrated rotometers, and flows were adjusted if they had changed by more than ± 5%. Samples were collected on preweighed 37-mm Teflon filters (Teflo; Gelman Sciences, Ann Arbor, MI). All filters were on- and off-weighed twice using a Mettler MT-5 Microbalance (Mettler Toledo International, Inc., Greifensee, Switzerland) in a temperature- and relative humidity-controlled weighing room after at least 48 hr equilibration time. These weights were used to determine filter loadings (Table 1).

Sample preparation and TNF bioassay. Detailed laboratory methods describing sample preparation and the TNF bioassay have been previously reported (*21–23,28,33*). Briefly, we cut filters into tiny pieces and placed them in a sterile, endotoxin-free saline solution for sonication. The filter pieces were removed after the sonication procedure, and an aliquot of the suspension was dried on a preweighed Teflo filter so that the mass recovery from the filters could be calculated (Table 1). Average percent recoveries were 59 ± 6% (range 22–88%) and 69 ± 7% (range 20–99%) for indoor and outdoor samples, respectively. Neither recoveries nor bioassay results are reported for the Home MAN1 samples; these samples were used in an earlier set of bioassays, so very little sample remained. UAP standard reference material 1649 (National Bureau of Standards, Washington, DC), which consists of total suspended particulates collected in the 1970s in Washington, D.C., was selected as a positive control. We dissolved UAP in saline solution at 10 mg/mL. All particle suspensions were kept frozen (–20°C) until use.

We harvested rat alveolar macrophages (AMs) from two female CD rats (250–300 g body weight, virus antibody free, Harlan Sprague Dawley, Inc., Indianapolis, IN) by bronchoalveolar lavage (BAL) using a phosphate-buffered saline (PBS) solution. After centrifugation, BAL cells were resuspended at 1×10^6 cells/mL in an assay buffer solution consisting of RPMI-1640 media (BioWhittaker, Walkersville, MD) supplemented with 1% fetal bovine serum (FBS), 0.1% balanced salt solution, penicillin, streptomycin, and L-glutamine (RPMI 1%). For the second set of priming experiments, AMs were initially treated with a 200 ng/mL (2,000 EU/mL) solution of bacterial lipopolysaccharides (*Escherichia coli* stereotype 0127:B8) at 37°C in humid 5% CO_2. Priming was done for 3 hr, and AMs were subsequently washed and resuspended in RPMI 1% at 2.4×10^6 cells/mL for use in the incubations.

Experimental incubations were completed in Ultra Low Cluster 96-well plates (Costar, Cambridge, MA), which were prepared according to manufacturer instructions. We performed three sets of incubations in duplicate (i.e., for the AMs from the two rats) for unprimed AMs: *a*) without particles (negative controls; $n = 4$); *b*) with UAP (positive controls; $n = 4$); and *c*) with indoor and outdoor particles ($PM_{2.5}$ samples; $n = 52$). Bioassays were repeated using primed AMs. We did not perform the TNF bioassay for either sample from Home MAN1 due to the extremely low recoveries. Briefly, we first dispensed 80 µL of assay buffer into each well. Next, we added 80 µL of either concentrated particle suspension (either indoor/outdoor $PM_{2.5}$ or UAP) or assay buffer (negative controls) to each test well; this was followed by 80 µL of cell suspension. Aliquots of each particle suspension were previously thawed, probe sonicated, and diluted so that a standard exposure concentration of 100 µg/mL was attained for each well. The plates were then incubated for 20 hr in a humidified incubator at 37°C with 5% CO_2. Upon completion, the well contents were placed on ice; an aliquot of supernatant was then removed and stored frozen for use in the TNF bioassay.

Table 1. Sampling locations and dates, and collected and recovered $PM_{2.5}$.

Home ID	Home location	Season	Starting date	Sampling duration (days)	Collected mass (µg)		Recovery (%)	
					Indoor	Outdoor	Indoor	Outdoor
MAN1	Manchester-by-the-Sea	Winter	13 Feb 1998	7	992.5	249.5	–	–
NEW1	Newton	Spring	26 Mar 1998	9	1387.5	1,457	22	39
		Fall	14 Oct 1998	7	940	828.5	87	74
WEL1	Wellesley	Spring	29 Apr 1998	11	2,420	1,568	68	20
		Winter	1 Dec 1998	7	1,583	819.5	51	82
SWP1	Swampscott	Summer	28 May 1998	8	1427.5	1442.5	56	49
BOX1	Boxford	Summer	9 Jun 1998	9	1,074	1211	35	41
		Winter	22 Nov 1998	7	397.5	561.5	83	95
NEW2	Newton	Summer	20 Jun 1998	6	2,100	2135.5	41	89
		Fall	23 Oct 1998	7	679.5	861	88	74
FOX1	Foxboro	Summer	7 Jul 1998	9	1273.5	1,317	50	93
		Winter	10 Oct 1998	7	579.5	1119.5	61	99
WEL2	Wellesley	Winter	5 Nov 1998	7	623.5	977.5	63	85
SWP2	Swampscott	Winter	13 Nov 1998	7	812.5	819.5	62	62
Mean ± SE					1,164 ± 56	1,098 ± 126	59 ± 5.5	69 ± 6.9

We conducted the TNF bioassay using a recently published microplate assay (*33*). This microplate assay uses a fluorescence-based quantification technique to assess TNF-induced cell death in the TNF-sensitive WEHI 164 clone 13 tumor cell line. Cell cultures were either dosed with AM supernatants or TNF standards (recombinant rat TNF; R&D Systems, Minneapolis, MN). We used a Cytofluor fluorescence plate reader (PerSeptive BioSystems, Inc., Framingham, MA) to measure propidium iodide fluorescence.

Endotoxin assay. Because other studies have shown that endotoxin is a potent stimulant of cytokine production (*17,23,25,28*), we also measured indoor as well as outdoor endotoxin concentrations for particle suspensions. Endotoxin was measured by *Limulus* assay (chromogenic *Limulus* amebocyte lysate kit; BioWhittaker) according to the manufacturer's instructions. Endotoxin concentrations are reported as endotoxin units (EU) per milligram of particles where 10 EU is equivalent to 1 ng of reference standard endotoxin. Endotoxin was measured for both whole particle suspensions and supernatants after centrifugation, but we report only data for the whole particle suspensions. Whole particle suspensions had significantly higher endotoxin concentrations than supernatants (2.62 ± 0.67 EU/mg versus 0.60 ± 0.08 EU/mg). This finding, which suggests that endotoxin predominantly exists in a particle-bound form, is similar to that of a previous study of concentrated ambient particles (CAPs) (*28*).

Measurement of particulate properties. As previously described in detail (*2*), we used state-of-the-art sampling methodologies to obtain a rich data set describing indoor and outdoor particles. We measured $PM_{2.5}$ indoors and outdoors using both 12-hr time-integrated HI samplers and continuous TEOM (tapered element oscillating microbalance) instruments (Model 1400A; Rupprecht & Patashnick Co., Inc., Albany NY). Real-time size distribution measurements were made using two particle sizing instruments, the scanning mobility particle sizer (SMPS, Model 3934; TSI, Inc., St. Paul, MN) and the aerodynamic particle sizer (APS, Model 3310A; TSI, Inc.). These instruments provided particle count concentrations within discrete size bins between 0.02 and 0.5 µm (SMPS) and 0.7 and 10 µm (APS). As described elsewhere (*2,7*), these instruments alternately sampled both indoor and outdoor air from ports in a specially designed stainless steel sampling manifold. Size distributions were obtained over 5-min sampling periods; indoor measurements were made at 0, 5, 10, 20, 25, 30, 40, 45, and 50 min after each hour and outdoor measurements were made at 15, 35, and 55 min after each hour. According to previously described methods (*2*), fine particle SMPS and APS data were converted to volume concentrations (cubic micrometers per cubic centimeter) for three particle size ranges: 0.02–0.1 µm ($PV_{0.02–0.1}$), 0.1–0.5 µm ($PV_{0.1–0.5}$), and 0.7–2.5 µm ($PV_{0.7–2.5}$).

In addition, 24-hr indoor and outdoor fine mass samples were collected on quartz fiber filters for elemental carbon/organic carbon (EC/OC) analysis. We used a parallel plate denuder containing carbon-impregnated papers for the spring–summer 1998 study homes to remove vapor-phase organic carbon before particle collection; use of this denuder was discontinued after extensive field testing indicated that the denuder efficiency was significantly < 100% (*34*). EC/OC samples were analyzed by thermal/optical reflectance (TOR) (*35*) at the Desert Research Institute (Reno, Nevada).

Other compositional data collected include total particle-bound polycyclic aromatic hydrocarbons (PAHs). PAHs were measured continuously using an Ecochem PAH monitor (Model 1002i; Ecochem Corporation, West Hills, CA), which sampled in 5-min intervals from the indoor/outdoor manifold. This instrument has been demonstrated to provide semiquantitative measurements through the photoelectric ionization of surface-bound PAHs (*6,36–38*). The instrument signal was output as a current (in units of picoamperes), but was approximately converted to a concentration (nanograms per cubic meter) using a universal conversion factor of 1 $\mu g/m^3/pA$ proposed by previous studies (*36,38*).

Other data collected during the comprehensive sampling activities include continuous air exchange rates and detailed time–activity information. Air exchange rates were measured in each home every 5 min using a sulfur hexafluoride tracer gas technique (*2,7,39*). This technique employs an SF_6 source that releases the gas into the home at a constant rate (6 mL/min) and a sensitive photoacoustic monitor that continuously measures the indoor SF_6 concentration (Model 3425; Brüel & Kjær, Nœrum, Denmark). Air exchange rates were computed using the 5-min SF_6 concentration data, the known source emission rate, and the home volume (*39*). Time–activity information was recorded by the home occupants in 20-min intervals using a daily time–activity diary.

Data analysis. We used Version 7 of the Statistical Analysis System (SAS Institute, Cary, NC) for all data analyses. Unless otherwise specified, all data are reported as means ± SEs. Data are presented as both TNF concentrations in picograms per milliliter and as endotoxin-normalized TNF concentrations in picograms per endotoxin unit. We computed endotoxin-normalized concentrations, which represent TNF releases for equivalent endotoxin levels, because of the well-known role of endotoxin as a stimulant of cytokine production (*17,23,25,28*). We did not use toxicity data from Home MAN1 in data analyses because of the absence of bioassay results.

We performed the following statistical analyses: descriptive statistics, nonparametric hypothesis tests, analysis of variance (ANOVA), Spearman correlations, and linear regressions. In all analyses, statistical significance was accepted for p-values < 0.05. We assessed differences between paired indoor/outdoor samples using a nonparametric Wilcoxon signed-rank test rather than a paired t-test because of the small sample size (n = 26 paired indoor/outdoor samples for the two rats). A two-way ANOVA analysis was performed to investigate the between-sample variability of indoor and outdoor data. In this analysis, rat was included as a blocking factor in the model to control for any variability in the TNF data that was due to differences in responses between the two rats. Spearman correlation coefficients were used to describe the relationship between indoor and outdoor particle and toxicity data. Particle data were all averaged over time periods (e.g., 6–12 days) matching the sampling times of the corresponding toxicity data. Linear regressions were performed to investigate the influence of endotoxin levels on the observed TNF responses.

Results

Particle characterization. As shown in Table 2, indoor and outdoor mean $PM_{2.5}$ concentrations for each week-long sampling period ranged between 4 and 27 $\mu g/m^3$ and were moderately correlated (R = 0.51; p = 0.06). Both indoor and outdoor mean $PM_{2.5}$ concentrations were highest during the July sampling event in Home NEW2 (26.6 and 27.5 $\mu g/m^3$, respectively). Relatively high mean indoor concentrations of 17.6 and 17.3 were also observed during the spring and winter sampling events in Home WEL1 despite lower ambient concentrations of 11.3 and 8.4 $\mu g/m^3$, respectively. As described by Long et al. (*2*), large indoor/outdoor differences such as these are due to the contributions of indoor source events such as cooking and cleaning activities. Both indoor and outdoor mean $PV_{0.1–0.5}$ and $PV_{0.7–2.5}$ concentrations were highly correlated (Spearman R = 0.72 and 0.91; p = 0.004 and < 0.0001, respectively; Table 2). However, the correlation for the $PV_{0.02–0.1}$ data was lower and insignificant (R = 0.51; p = 0.06), and in contrast to $PV_{0.1–0.5}$ and $PV_{0.7–2.5}$ data, indoor $PV_{0.02–0.1}$ concentrations on average were greater than outdoor

concentrations for these study homes. These findings again reflect the impacts of indoor source events, which have been shown to be more pronounced for ultrafine particles (*2*).

As described previously (*2*), indoor mean organic carbon concentrations were significantly larger than outdoor concentrations (means of 7.8 and 3.0 μg/m^3, respectively), suggesting that indoor particle events may be important sources of indoor organic carbon. Due to the impact of indoor organic carbon sources, there was very little correlation between indoor and outdoor concentrations (R = 0.02; Table 2). In contrast, mean indoor and outdoor elemental carbon concentrations were very similar (0.88 and 0.99 μg/m^3, respectively) and highly correlated (R = 0.77; p = 0.0014). Indoor and outdoor mean PAH concentrations were also extremely well correlated (R = 0.99; p < 0.0001), suggesting that there were few important indoor PAH sources in the study homes.

Although not statistically significant, indoor endotoxin levels were on average higher than outdoor levels (Figure 1, Tables 2 and 3). The indoor and outdoor mean endotoxin concentrations were 3.3 ± 1.3 and 2.0 ± 4 EU/mg, respectively. The maximum endotoxin concentration was 18.1 EU/mg for the indoor sample from the spring sampling event in Home NEW1, whereas the corresponding outdoor concentration was 5.0 EU/mg (Table 3). The indoor/outdoor correlation was low (R = 0.18; p = 0.57) and the median indoor:outdoor ratio for matching data from each home was 1.5, both suggesting the potential importance of indoor endotoxin sources. Despite the small number of samples (n = 13), outdoor endotoxin concentrations were significantly higher (p = 0.007) in the two homes sampled in the spring, which might be due to elevated plant emissions during the growing season (*40*).

Overview of indoor versus outdoor toxicity responses. Figure 2 shows a comparison of the TNF release of unprimed AMs for the indoor and outdoor $PM_{2.5}$ samples as well as the negative and positive controls. Table 3 summarizes TNF releases of unprimed AMs by house and season where data have been averaged for the two rats.

We detected TNF only in one of four negative controls (at 14 pg/mL), whereas TNF releases for indoor and outdoor samples were on average > 200 and > 100 pg/mL, respectively. On average, the response of the indoor samples was just slightly higher than that for the UAP positive controls (279 ± 78 pg/mL vs. 270 ± 54 pg/mL). When indoor and outdoor data were normalized for endotoxin levels, the mean indoor response was still nearly twice as high as the mean outdoor response (952 ± 157 and 494 ± 96 pg/EU, respectively). The disparity between indoor/outdoor data remained for the endotoxin-normalized data despite the fact that normalization for endotoxin levels changed the relative ranks of many of the data (Table 3). For example, the average TNF release for the spring NEW1 indoor sample (1,524 pg/mL) was nearly 3 times greater than the next largest response for the unadjusted data. After normalization for endotoxin, it was reduced to a value (840 pg/EU) less than the indoor mean.

For both the unadjusted and endotoxin-normalized data, Wilcoxon signed-rank tests of paired indoor and outdoor samples showed that indoor TNF releases were significantly higher than the corresponding outdoor data (p = 0.045 and 0.01, respectively). Indoor–outdoor correlations confirmed the poor relationship between indoor and outdoor data (Figure 3). In contrast to such particulate properties as elemental carbon, PAHs, $PV_{0.1–0.5}$, and $PV_{0.7–2.5}$ (Table 2), there was little correlation between indoor and outdoor toxicity responses. Nonsignificant correlations of –0.20 (p = 0.51) were obtained for both unadjusted and endotoxin-normalized data. As mentioned above, low and insignificant correlations were also observed for organic carbon and endotoxin, each of which has been demonstrated to have important indoor sources (*2,40–44*).

As shown by Figures 2 and 3, indoor TNF releases were also more highly variable than outdoor TNF releases. A two-way ANOVA model, which included rat as a blocking factor to control for variability in TNF releases among individual rats, was used to statistically test the sample-to-sample variability of indoor and outdoor samples. Although several homes were sampled twice in different seasons, each sample was treated as an independent sample because ambient and home conditions differed between the two sampling seasons in the same home. Despite the small sample size, the variability of both the indoor-unadjusted and endotoxin-normalized data was found to be statistically significant (p < 0.0001 and 0.04, respectively). However, for both sets of outdoor data, the between-sample variability was insignificant (p = 0.27 and 0.49 for the unadjusted and endotoxin-normalized data, respectively). These findings for the outdoor data suggest that the significant indoor between-sample variability is not due to differences in ambient particle toxicity. Instead, given that each bioassay was conducted for the same particle concentration (100 μg/mL), differences in indoor particle properties such as composition are likely responsible for the observed indoor between-sample variability.

Similar to previous studies (*23,29*), TNF production was significantly elevated for lipopolysaccharide-primed cells, both for negative controls as well as for particle suspensions (data not shown). For the negative controls, priming elicited a mean response of 1,302 ± 349 pg/mL, which is three orders of magnitude higher than that for the unprimed controls. The priming effect was even more

Table 2. Summary statistics for indoor/outdoor particulate data.[a]

Parameter	No.	Location	Particulate concentration data Mean ± SE	Min	Median	Max	Indoor/outdoor correlations Spearman R	p-Value
$PM_{2.5}$ (μg/m^3)	14	In	11.8 ± 1.9	5.7	10.7	26.6	0.51	0.06
		Out	11.1 ± 1.5	4.1	10.3	27.5		
$PV_{0.02–0.1}$ (μm^3/cm^3)	14	In	0.68 ± 0.10	0.18	0.54	1.4	0.51	0.06
		Out	0.48 ± 0.05	0.23	0.49	0.85		
$PV_{0.1–0.5}$ (μm^3/cm^3)	14	In	6.2 ± 0.90	1.7	6.2	14.7	0.72	0.004
		Out	6.3 ± 0.94	2.1	5.8	15.0		
$PV_{0.7–2.5}$ (μm^3/cm^3)	13[b]	In	2.3 ± 0.45	0.85	1.9	6.5	0.91	< 0.0001
		Out	2.7 ± 0.49	1.1	2.0	8.0		
EC (μg/m^3)	14	In	0.88 ± 0.09	0.35	0.90	1.5	0.77	0.0014
		Out	0.99 ± 0.10	0.47	0.96	1.6		
OC (μg/m^3)	14	In	7.8 ± 0.57	4.7	7.8	13.6	0.02	0.95
		Out	3.0 ± 0.19	1.4	3.2	4.2		
PAH (ng/m^3)	14	In	31 ± 5.6	4.2	25	82	0.99	< 0.0001
		Out	37 ± 7.7	5.6	27	107		
Endotoxin (EU/mg)	13[c]	In	3.3 ± 1.3	0.51	2.2	18.1	0.18	0.57
		Out	2.0 ± 0.41	0.36	1.5	5.0		

Abbreviations: EC, elemental carbon; Max, maximum; Min, minimum; OC, organic carbon.
[a]All concentrations and correlations are for data that have been averaged over the duration of the sampling period within a home to match the sampling duration of the corresponding toxicity samples. [b]$PV_{0.7-2.5}$ data are not available for the spring sampling event in Home NEW1 due to instrument failure. [c]Endotoxin data are not reported for Home MAN1 due to extremely low sample recoveries (see text).

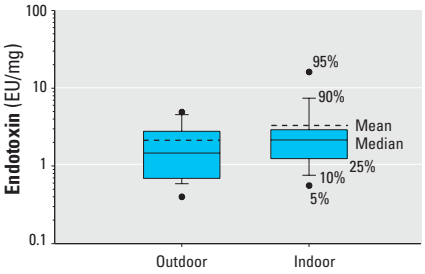

Figure 1. Box plots comparing outdoor and indoor endotoxin levels.

amplified in the presence of particles, as the average indoor and outdoor primed responses were over 3,500 and 2,500 pg/mL, respectively. These results demonstrate the enhanced sensitivity of primed cells to both indoor and outdoor fine particles, which has been shown previously for CAP samples (*23,29*). Given the similarity of the indoor/outdoor relationship for the unprimed and primed data, our focus for the remainder of this paper is on the unprimed bioassay data.

Evidence of endotoxin-induced TNF production. Figure 4A and B shows the relationship between endotoxin concentrations and TNF responses for the indoor and outdoor data. These plots show that endotoxin levels were strongly associated with TNF responses for both indoor and outdoor fine particle data. The indoor regression was performed with and without the extreme data point for the spring Home NEW1 sample. This sample yielded the highest endotoxin concentration (18.1 EU/mg) as well as the highest mean TNF responses for both unprimed and primed AMs (over 1,500 and 15,000 pg/mL, respectively). Despite the fact that this data point highly influenced the model fit (as indicated by the R^2 values of 0.92 and 0.40 with and without this data point, respectively), it did not bias the fitted slope which just slightly dropped from 84 to 82 when the data point was removed.

Although endotoxin levels explained a similar level of variability in TNF releases for both indoor and outdoor data (R^2 values were both approximately 0.40 when the indoor NEW1 data were excluded), the regression slopes differed for the two sets of data. As mentioned above, the indoor slope was approximately 80; however, the outdoor slope was only 49. The difference between the two slopes suggests that the magnitude of the endotoxin-mediated toxicity response may depend on other particle properties. The synergistic interactions between endotoxin and other proinflammatory components of environmental particles have been previously hypothesized (*23,28*). In a series of bioassay experiments, Imrich et al. (*23*) demonstrated that there was no difference in TNF release between primed cells treated with saline (control) or with inert TiO_2 particles. However, TNF releases were highly amplified when primed cells were treated with CAPs (*23*). Furthermore, Ning et al. (*28*) showed that particle-associated endotoxin in CAP samples elicits much greater bioactivity than the same amount of soluble endotoxin given to AMs alone. For this study, the larger indoor slope suggests that there is greater synergism between endotoxin and components of indoor particles.

Influence of air exchange rate on indoor particle toxicity. Figure 5A and B shows that higher indoor TNF releases and indoor/outdoor differences in TNF releases were typically observed in homes with lower air exchange rates, particularly for the endotoxin-normalized data. Air exchange rates were classified as either high or low on the basis of whether they were above or below the median home air exchange rate of 0.84/hr. Despite the small sample size, both indoor endotoxin-normalized TNF releases, as well as the difference between indoor/outdoor endotoxin-normalized TNF releases, were significantly higher for the low air exchange rate class (p = 0.005 for both). Similar Wilcoxon ranked-sum tests of the unadjusted data yielded insignificant p-values of 0.23 and 0.14, respectively. These findings, together with evidence that the impacts of indoor source events are even more pronounced at low air exchange rates when indoor residence times are longer and indoor-generated particles can accumulate (*2,7,45*), suggest that the higher indoor toxicity responses may be due to the effects of indoor-generated particles.

Estimation of indoor toxicity components. We constructed a simple physical–statistical model to quantify the relative contributions of indoor-generated and ambient particles to the indoor toxicity response. This model assumes that the endotoxin-normalized indoor toxicity response (Tox'_{in} in picograms per endotoxin unit) is a function of the fraction of particles of indoor origin (F_{in}) and those of ambient origin (F_a):

$$Tox'_{in} = \alpha_{in}F_{in} + \alpha_a F_a, \qquad (1)$$

where α_{in} and α_a (both in picograms per endotoxin unit) represent the portions of the indoor toxicity response that can be attributed to particles of indoor origin and those of ambient origin, respectively. This model is based on the fact that each bioassay was conducted for a uniform exposure concentration of 100 µg/mL, thus effectively removing any relationship with particle concentration. We also assumed that F_{in} and F_a represent the fraction of indoor-generated and ambient particles, respectively, in the 100 µg/mL particle suspensions. We used endotoxin-normalized data in the model because we hypothesized that there are synergistic interactions between particles and endotoxin which depend on particle properties.

Table 3. Summary of $PM_{2.5}$ endotoxin and toxicity data by house and season.

		Endotoxin concentration (EU/mg)		TNF release[a]			
				Unadjusted (pg/mL)		Normalized (pg/EU)	
Home ID	Season	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
MAN1	Winter	—	—	—	—	—	—
NEW1	Spring	18.135	4.989	1,524	64	840	127
	Fall	2.018	1.463	221	41	1,093	277
WEL1	Spring	1.049	4.477	55	389	520	869
	Winter	1.304	0.937	218	18	1,668	192
SWP1	Summer	2.465	2.861	71	87	288	302
BOX1	Summer	4.737	0.648	558	25	1,177	378
	Winter	2.896	2.182	135	209	466	958
NEW2	Summer	0.819	0.355	26	7	318	197
	Fall	2.998	2.056	37	257	123	1,250
FOX1	Summer	1.368	0.678	101	18	735	258
	Winter	2.179	0.698	324	37	1,485	530
WEL2	Winter	0.511	2.748	134	178	2,613	646
SWP2	Winter	2.16	1.355	227	60	1,051	443
Mean ± SE		3.3 ± 1.3	2.0 ± 0.1	279 ± 111	107 ± 32	952 ± 190	494 ± 95

[a]Data are mean values for test results for two rats.

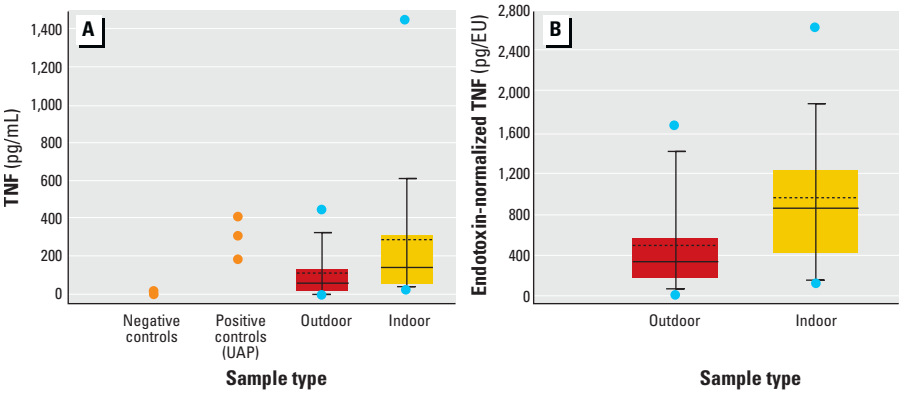

Figure 2. TNF production of unprimed AMs in negative controls (n = 4), positive controls (UAP; n = 4), and indoor (n = 26) and outdoor (n = 26) $PM_{2.5}$ samples. (*A*) Unadjusted TNF data. (*B*) Endotoxin-normalized TNF data.

Because $F_a = 1 - F_{in}$, Equation 1 is simply equal to the following:

$$Tox'_{in} = \alpha_{in}F_{in} + \alpha_a(1 - F_{in}) \quad (2)$$

After rearranging terms, the following equation is obtained:

$$Tox'_{in} = (\alpha_{in} - \alpha_a)F_{in} + \alpha_a \quad (3)$$

If Tox'_{in} is regressed on F_{in}, the slope (e.g., $\alpha_{in} - \alpha_a$) approximates the difference between the mean endotoxin-normalized TNF response attributable to indoor-generated particles and that for indoor particles of ambient origin, whereas the intercept (e.g., α_a) represents the mean response due to indoor particles of ambient origin.

We calculated model values for F_{in} for these study homes using previously reported estimates of ambient particle infiltration factors (F_{INF}) (*46*). Infiltration factors, which ranged from 0.40 to 1.09, were estimated from simultaneous indoor/outdoor $PM_{2.5}$ data from nighttime, nonsource periods for all but the spring sampling events in Homes WEL1 and NEW1 (*46*). Nightly F_{INF} estimates were averaged over the entire sampling duration within a home to match the averaging period of the toxicity data. For the spring sampling events in Homes WEL1 and NEW1, where matching indoor/outdoor continuous $PM_{2.5}$ measurements were not available, SMPS and APS particle volume data were summed to approximate $PV_{2.5}$. The indoor concentration of ambient fine particles (C_a) was first quantified by multiplying the infiltration factor by the outdoor $PM_{2.5}$ concentration (C_{out}):

$$C_a = F_{INF} \times C_{out} \quad (4)$$

It was then possible to estimate the indoor fraction of ambient particles

$$F_a = \frac{C_a}{C_{in}} \quad (5)$$

and the corresponding indoor fraction of indoor-generated particles

$$F_{in} = 1 - F_a \quad (6)$$

Estimates of F_{in} ranged from a low of approximately 0 for the summer sampling events in Homes SWP1, BOX1, and NEW1 to a high of 0.74 for the winter sampling event in Home WEL1 (0.28 ± 0.06, mean ± SE).

Model results presented in Figure 6A and B suggest the enhanced bioactivity of indoor-generated particles. Despite the small data set, we found a strong and near-significant relationship (R^2=0.29; p = 0.06) between the endotoxin-normalized TNF release and F_{in} (Figure 6A). Furthermore, the intercept of 491 ± 275 pg/EU, which represents the mean endotoxin-normalized TNF response attributable to indoor particles of ambient origin, was very close to the outdoor mean TNF release of 494 ± 96 pg/EU. In contrast, the estimate for the mean endotoxin-normalized TNF response attributable to indoor-generated particles was approximately 2,100 ± 600 pg/EU (Figure 6B).

Discussion

Rat AMs treated with either indoor or outdoor $PM_{2.5}$ released significant amounts of TNF compared to control AMs. Furthermore, these TNF releases for both indoor and outdoor $PM_{2.5}$ samples are of similar magnitude to that observed for CAPs by other investigators. Specifically, Imrich et al. (*23*) observed mean TNF releases of 10–130 pg/mL for rat AMs exposed to 100 µg/mL CAPs from several daily samples, and 20–260 pg/mL for human alveolar macrophages exposed to 50 µg/mL CAP suspensions. In addition, TNF production was further amplified for primed AMs exposed to these indoor and outdoor $PM_{2.5}$ samples. This priming effect is comparable to that observed by a previous study of rat and human AMs exposed to CAPs or UAP (*23*). These results suggest that indoor $PM_{2.5}$ may also have a synergistic effect on the inflammatory response in people with preexisting proinflammatory conditions.

A comparison of paired indoor/outdoor data demonstrated that significantly greater TNF releases were elicited by indoor $PM_{2.5}$ samples than by the corresponding outdoor samples. The significance of this indoor/outdoor difference slightly increased when data were normalized for endotoxin concentrations. This finding alone suggests that indoor particles are at least as toxic as outdoor particles. As described earlier, indoor particles include particles of both ambient and indoor origin. Together with this indoor/outdoor difference, other study findings suggest that particles of indoor origin may be more bioactive than particles of ambient origin.

The role of indoor-generated particles in indoor particle bioactivity is supported by several study findings. Specifically, the low indoor/outdoor correlation between paired toxicity data is suggestive of the impact of indoor-generated particles. Also, regressions of TNF releases on endotoxin concentrations yielded steeper slopes for indoor than outdoor data, suggesting that there may be greater synergism between endotoxin and components of indoor particles. In addition, indoor but not outdoor TNF releases were

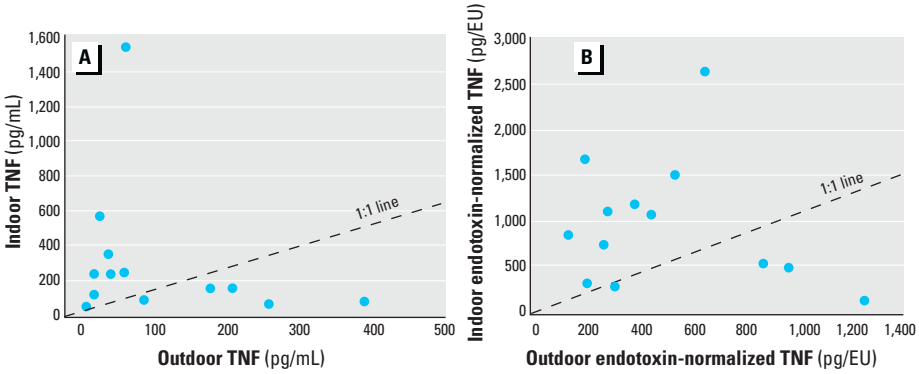

Figure 3. Indoor versus outdoor TNF releases for (*A*) unadjusted data (Spearman R = –0.20; p = 0.51; n = 13) and (*B*) endotoxin-normalized data (Spearman R = –0.20; p = 0.51; n = 13). Each data point represents data from one sampling event in which TNF releases have been averaged over the two rats (n = 13).

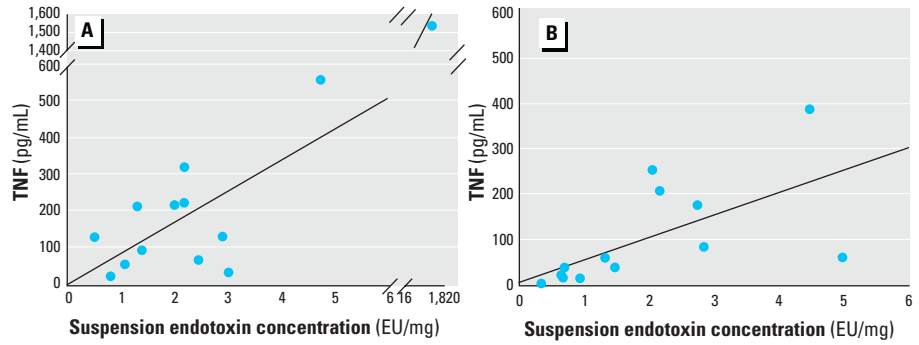

Figure 4. Linear regressions of TNF release versus endotoxin concentration for (*A*) indoor data [regression output: slope = 84 (p < 0.01); r^2 = 0.92; without extreme data point: slope = 82 (p = 0.03); r^2 = 0.40)] and (*B*) outdoor data [regression output: slope = 49 (p = 0.02); r^2 = 0.38].

shown to exhibit significant between-sample variability. Due to the use of a uniform exposure concentration of 100 µg/mL, the variability in indoor TNF releases may be attributed to fluctuations in particle properties (e.g., composition, size) among the indoor samples. The contributions of indoor source events have been previously shown to be a dominant source of variability for indoor particle concentrations and size distributions (*2,7*). Furthermore, it has been previously reported that source strengths of indoor particle events were highly variable in these study homes (*2*). If differences in indoor source types and event frequency among the study homes are also considered, it is likely that variability in indoor particle emission rates, as well as particle characteristics among the study homes, may explain the variability in indoor TNF releases.

It has also been reported that the impacts of indoor particle events are amplified under conditions of low air exchange rate (*2,7,45*). Results from the present study have shown that the level of indoor bioactivity and the difference between indoor and outdoor bioactivities depend on air exchange rate. The five largest indoor/outdoor differences in the endotoxin-normalized TNF release occurred for homes with lower exchange rates (i.e., below the study median), suggesting that the indoor toxicity response is amplified when air exchange rates are low and indoor residence times are high. During low air exchange rate conditions, indoor particle events can dramatically increase the fraction of indoor particles of indoor origin as concentrations of indoor-generated particles build up. In addition, low air exchange rates are also associated with decreased ambient particle infiltration (*46–49*), which results in diminished indoor concentrations of ambient particles. Thus, it would appear that conditions which favor the accumulation of indoor-generated particles rather than ambient particles may thus raise the toxicity of indoor particles.

Despite the small sample size, the results of our simple physical–statistical model confirm that indoor particle toxicity may be elevated as the fraction of indoor-generated particles increases. This model demonstrated that differences in the indoor toxicity response between samples could be explained by the fractions of particles of indoor and ambient origin. The mean endotoxin-normalized TNF response attributable to particles of indoor origin was over four times higher than the corresponding estimate for particles of ambient origin (2,100 ± 600 pg/EU versus 491 ± 275 pg/EU).

It is still unclear which components of indoor-generated particles may be responsible for their enhanced bioactivity. In this study we have confirmed the role of endotoxin as a stimulant of cytokine production (*17,23,25,28*). However, endotoxin levels were not found to differ significantly between indoor and outdoor fine particles. In addition, normalization for endotoxin did not eliminate the variability in the indoor TNF data, suggesting that there are other proinflammatory components of indoor particles. Based on *in vitro* experiments employing endotoxin inhibitors, other investigators have hypothesized that there are other proinflammatory components of ambient particles (*28,29*). One possible proinflammatory component for indoor particles may be organic carbon, which was present in significantly higher concentrations in indoor $PM_{2.5}$ samples. Organic carbon is known to be enriched in fine particles, and previous studies have demonstrated the mutagenic (*50–52*) and carcinogenic (*53*) properties of airborne particulate-bound carbon.

Although these results are suggestive, caution must be exercised in interpreting them. The implications of these results with respect to *in vivo* effects are very uncertain. *In vitro* exposure conditions are clearly not representative of particle inhalation and deposition in the lungs. In addition, although TNF is known to initiate the inflammatory activation of AMs, it is unclear whether the differences in TNF release for indoor versus outdoor $PM_{2.5}$ would result in different *in vivo* toxic effects. Furthermore, these study findings are also based on a small sample size. $PM_{2.5}$ samples were collected from only nine homes and represent very small periods of time in these homes (e.g., 1–2 weeks).

The intent of this study was to explore the relative toxicities of indoor and outdoor fine particles. These study findings indicate that particles of indoor origin can induce cytokine production, and they point to the need for additional efforts to understand indoor exposures to both particles of indoor origin as well as those of ambient origin. Given the large amounts of time that people spend indoors, these study findings suggest that indoor particles should be the focus of further toxicologic research.

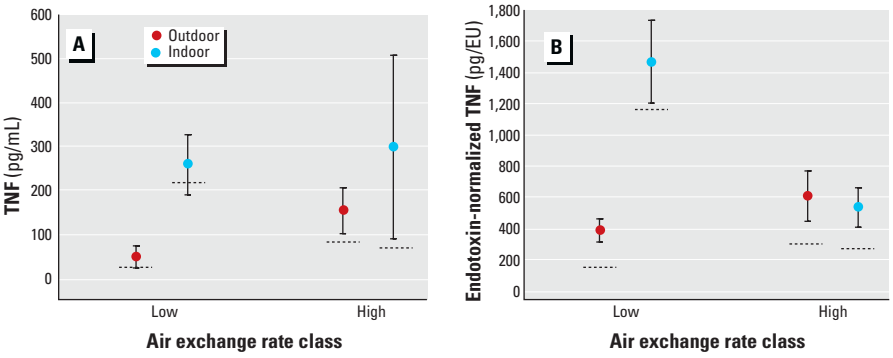

Figure 5. Indoor and outdoor TNF releases for (*A*) unadjusted and (*B*) endotoxin-normalized data stratified by air exchange rate (e.g., high, above the study median, $n = 6$; low, below the study median, $n = 7$). Circles represent means, error bars are SEs, and dotted lines indicate medians.

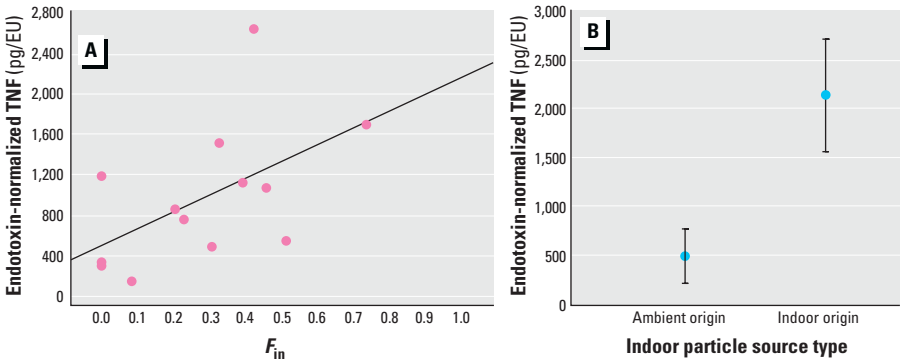

Figure 6. Results of the simple physical–statistical model used to apportion indoor endotoxin-normalized TNF releases attributable to particles of ambient versus indoor origin. (*A*) Regression of the indoor endotoxin-normalized TNF release on the F_{in} {coefficients: b[0] = 491 ± 275 ($p = 0.10$); b[1] = 1,624 ± 769 ($p = 0.06$); $r^2 = 0.29$}. (*B*) Comparison of model estimates (means ± SE) for the bioactivity of particles of ambient versus indoor origin.

References and Notes

1. U.S. EPA. Air Quality Criteria for Particulate Matter. Research Triangle Park, NC:U.S. Environmental Protection Agency, Office of Research and Development, 1996.
2. Long CM, Suh HH, Koutrakis P. Characterization of

indoor particle sources using continuous mass and size monitors. J Air Waste Manag Assoc 50:1236–1250 (2000).
3. Kamens R, Lee C-T, Weiner R, Leith D. A study to characterize indoor particles in three non-smoking homes. Atmos Environ 25A:939–948 (1991).
4. Koutrakis P, Briggs SLK, Leaderer BP. Source apportionment of indoor aerosols in Suffolk and Onondaga counties, New York. Environ Sci Technol 26:521–527 (1992).
5. Özkaynak H, Xue J, Spengler J, Wallace L, Pellizzari E, Jenkins P. Personal exposure to airborne particles and metals: results from the Particle Team Study in Riverside, California. J Exp Anal Environ Epidemiol 6:57–78 (1996).
6. Wallace L, Quakenboss J, Rodes C. Continuous measurements of particles, PAH, and CO in an occupied townhouse in Reston, VA. In: Measurement of Toxic and Related Air Pollutants: Proceedings of a Speciality Conference, Research Triangle Park, NC, 29 April–1 May 1997. Pittsburg, PA:Air & Waste Management Association, 1997;860–871.
7. Abt E, Suh HH, Allen G, Koutrakis P. Characterization of indoor particle sources: a study conducted in the metropolitan Boston area. Environ Health Perspect 108:35–44 (2000).
8. Michaels RA, Kleinman MT. Incidence and apparent health significance of brief airborne particle excursions. Aerosol Sci Technol 32:93–105 (2000).
9. Gold DR, Litonjua A, Schwartz J, Lovett E, Larson A, Nearing B, Allen G, Verrier M, Cherry R, Verrier R. Ambient pollution and heart rate variability. Circulation 101:1267–1273 (2000).
10. Peters A, Liu E, Verrier RL, Schwartz J, Gold DR, Mittleman M, Baliff J, Oh JA, Allen G, Monahan K, et al. Air pollution and incidence of cardiac arrhythmia. Epidemiology 11:11–17 (2000).
11. Delfino RJ, Zeiger RS, Seltzer JM, Street DH. Symptoms in pediatric asthmatics and air pollution: differences in effects by symptom severity, anti-inflammatory medication use and particulate averaging time. Environ Health Perspect 106:751–761 (1998).
12. Morgan G, Corbett S, Wlodarczyk J. Air pollution and hospital admissions in Sydney, Australia, 1990–1994. Am J Public Health 88:1761–1766 (1998).
13. Korrick SA, Neas LM, Dockery DW, Gold DR, Allen GA, Hill LB, Kimball KD, Rosner BA, Speizer FE. Effects of ozone and other pollutants on the pulmonary function of adult hikers. Environ Health Perspect 106:93–99 (1998).
14. Pope CA. Review: epidemiological basis for particulate air pollution health standards. Aerosol Sci Technol 32:4–14 (2000).
15. Godleski JJ, Verrier RL, Koutrakis P, Catalano P. Mechanisms of Morbidity and Mortality from Exposure to Ambient Air Particles. Research Report 91. Cambridge, MA:Health Effects Institute, 2000.
16. Gordon T, Nadziejko C, Chen LC, Schlesinger R. Effects of Concentrated Ambient Particles in Rats and Hamsters: An Exploratory Study. Research Report 93. Cambridge, MA:Health Effects Institute, 2000.
17. Dong W, Lewtas J, Luster MI. Role of endotoxin in tumor necrosis factor α expression from alveolar macrophages treated with urban air particles. Exp Lung Res 22:577–592 (1996).
18. Becker S, Soukup JM, Gilmour MI, Devlin RB. Stimulation of human and rat alveolar macrophages by urban air particulates: effects on oxidant radical generation and cytokine production. Toxicol Appl Pharmacol 141:637–648 (1996).
19. Yang H-M, Ma JYC, Castranova V, Ma JKH. Effects of diesel exhaust particles on the release of interleukin-1 and tumor necrosis factor-alpha from rat alveolar macrophages. Exp Lung Res 23:269–284 (1997).
20. Carter JD, Ghio AJ, Samet JM, Devlin RB. Cytokine production by human airway epithelial cells after exposure to an air pollution particle is metal-dependent. Toxicol Appl Pharmacol 146:180–188 (1997).
21. Goldsmith C-A, Frevert C, Imrich A, Sioutas C, Kobzik L. Alveolar macrophage interaction with air pollution particulates. Environ Health Perspect 105(suppl 5):1191–1195 (1997).
22. Goldsmith C-AW, Imrich A, Danaee H, Ning Y, Kobzik L. Analysis of air pollution particulate-mediated oxidant stress in alveolar macrophages. J Toxicol Environ Health 54A:101–117 (1998).
23. Imrich A, Ning YY, Koziel H, Coull B, Kobzik L. Lipopolysaccharide priming amplifies lung macrophage tumor necrosis factor production in response to air particles. Toxicol Appl Pharmacol 159:117–124 (1999).
24. Drumm K, Schindler H, Buhl R, Küstner E, Smolarski R, Kienast K. Indoor air pollutants stimulate interleukin-8-specific mRNA expression and protein secretion of alveolar macrophages. Lung 177:9–19 (1999).
25. Elder ACP, Gelein R, Finkelstein JN, Cox C, Oberdörster G. Endotoxin priming affects the lung response to ultrafine particles and ozone in young and old rats. Inhal Toxicol 12:85–98 (2000).
26. Monn C, Becker S. Cytotoxicity and induction of proinflammatory cytokines from human monocytes exposed to fine ($PM_{2.5}$) and coarse particles ($PM_{10-2.5}$) in outdoor and indoor air. Toxicol Appl Pharmacol 155:245–252 (1999).
27. Frampton MW, Ghio AJ, Samet JM, Carson JL, Carter JD, Devlin RB. Effects of aqueous extracts of PM_{10} filters from the Utah Valley on human airway epithelial cells. Am J Physiol 277:L960–L967 (1999).
28. Ning Y, Imrich A, Goldsmith C-A, Qin G, Kobzik L. Alveolar macrophage cytokine production in response to air particles in vitro: role of endotoxin. J Toxicol Environ Health 59A:165–180 (2000).
29. Imrich A, Ning Y, Kobzik L. Insoluble components of concentrated air particles mediate alveolar macrophage responses in vitro. Toxicol Appl Pharmacol 167:140–150 (2000).
30. Driscoll KE. TNFα and MIP-2: role in particle-induced inflammation and regulation by oxidative stress. Toxicol Lett 112–113:177–184 (2000).
31. Marple VA, Rubow KL, Turner W, Spengler JD. Low flow rate sharp cut impactors for indoor air sampling: design and calibration. JAPCA 37:1303–1307 (1987).
32. Lioy PJ, Wainman T, Turner W, Marple VA. An intercomparison of the indoor air sampling impactor and the dichotomous sampler for a 10-µm cut size. JAPCA 38:668–670 (1988).
33. Imrich A, Taylor M, Kobzik L. Fluorescence-based microplate bioassay for tumor necrosis factor. J Immunol Methods 212:109–112 (1998).
34. Van Loy M, Saxena P, Babich P, Lawrence J, Allen G, Koutrakis P. Unpublished data.
35. Chow JC, Watson JG, Pritchett LC, Pierson WR, Frazier CA, Purcell RG. The DRI thermal/optical reflectance carbon analysis system: description, evaluation, and applications in U.S. air quality studies. Atmos Environ 27A:1185–1201 (1993).
36. Wilson NK, Barbour RK, Chuang JC, Mukund R. Evaluation of a real-time monitor for fine particle-bound PAH in air. Polycycl Aromat Comp 5:167–174 (1994).
37. Chuang JC, Ramamurthi M. Evaluation of a Gossen, GmbH Model PAS 1001i Photoelectric Aerosol Sensor for Real-Time PAH Monitoring. Research Triangle Park, NC:U.S. Environmental Protection Agency, National Exposure Research Laboratory, 1997.
38. Dubowsky SD, Wallace LA, Buckley TJ. The contribution of traffic to indoor concentrations of polycyclic aromatic hydrocarbons. J Exp Anal Environ Epidemiol 9:312–321 (1999).
39. Lebret E, Boleij J, Brunekreef B. Home ventilation under normal living conditions. In: Indoor Air '90: Proceedings of the Fifth International Conference on Indoor Air Quality and Climate, Toronto, July 19–August 3, 1990. Ottawa, Ontario, Canada:International Conference on Indoor Air Quality and Climate, 1990;413–418.
40. Park J-H. Endotoxin in the Home: Exposure Assessment and Health Effect [PhD Dissertation]. Boston:Harvard School of Public Health, 1999.
41. Rogge WF, Hildemann LM, Mazurek MA, Cass GR, Simoneit BRT. Sources of fine organic aerosol. 1. Charbroilers and meat cooking operations. Environ Sci Technol 25:1112–1125 (1991).
42. Rogge WF, Hildemann LM, Mazurek MA, Cass GR, Simoneit BRT. Sources of fine organic aerosol. 5. Natural-gas home appliances. Environ Sci Technol 27:2736–2744 (1993).
43. Rogge WF, Hildemann LM, Mazurek MA, Cass GR, Simoneit BRT. Sources of fine organic aerosol. 9. Pine, oak and synthetic log combustion in residential fireplaces. Environ Sci Technol 32:13–22 (1998).
44. Allen AG, Miguel AH. Indoor organic and inorganic pollutants: in-situ formation and dry deposition in southeastern Brazil. Atmos Environ 29:3519–3526 (1995).
45. Tu K-W, Knutson EO. Indoor outdoor aerosol measurements for two residential buildings in New Jersey. Aerosol Sci Technol 9:71–82 (1988).
46. Long CM, Suh HH, Catalano PJ, Koutrakis P. Using time- and size-resolved particulate data to quantify penetration and deposition behavior. Environ Sci Technol 35:2089–2099 (2001).
47. Wallace LA. Indoor particles: a review. J Air Waste Manag Assoc 46:98–126 (1996).
48. Wilson WE, Suh HH. Fine particles and coarse particles: concentration relationships relevant to epidemiological studies. Air Waste Manag Assoc 47:1238–1249 (1997).
49. Wilson WE, Mage DT, Grant LD. Estimating separately personal exposure to ambient and non-ambient particulate matter for epidemiology and risk assessment: why and how. Air Waste Manag Assoc 50:1167–1183 (2000).
50. Löfroth G, Stensman C, Brandhorst-Satzkorn M. Indoor sources of mutagenic aerosol particulate matter: smoking, cooking and incense burning. Mutat Res 261:21–28 (1991).
51. Nardini B, Granella M, Clonfero E. Mutagens in indoor air particulate. Mutat Res 322:193–202 (1994).
52. Pedersen DU, Durant JL, Penman BW, Crespi CL, Hemond HF, Lafleur AL, Cass GR. Seasonal and spatial variations in human cell mutagenicity of respirable airborne particles in the northeastern United States. Environ Sci Technol 33:4407–4415 (1999).
53. Lewtas J. Complex mixtures of air pollutants: characterizing the cancer risk of polycyclic organic matter. Environ Health Perspect 100:211–218 (1993).